With Exposure to Mercury, there is a Lesser Amount of Movement of Growth Cones of Chick Sympathetic Neurons in the Experimental Sample than in the Control Sample as Measured by a Change in Degree

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Introduction:

In this study I tested the hypothesis that there will be a greater amount of movement of the growth cones in chick sympathetic neurons without being exposed to mercury, then there would be with mercury exposure. The control group will be growth cones exposed to an L15 buffer and the experimental group will be growth cones exposed to mercuric chloride ($HgCl_2$). This hypothesis will be tested by measuring a change, if any, in the degree of two sets of diverging filopodia in the experimental/post Hg, and two sets of diverging filopodia in the control/post buffer. The degree will be measured by finding the angle between two filopodia that are side by side in two different locations on the neuron. Then the change in degree will be observed between those two filopodia in two-minute increments for eight minutes. This will be measured on both the control and experimental groups, and then will be compared.

Growth cones are found at the tip of neurons and play an important role in the development of the neuron. Two principle proteins involved in growth cone structure and function are actin and tubulin (Zernicke, 1982). During cell growth, actin is responsible for motion and tubulin molecules link together and form microtubules, which are a structural component of the axon (Ngim, 1992). With exposure to mercury, the processes of actin and tubulin are interrupted (Ngim, 1992). I believe that there will be more evidence of growth cone movement before mercury exposure because when using time-lapse photography with microscopy, it has been observed that within a few minutes of exposure to mercury, growth cones lost their motility and exhibited collapse and retraction (Leong et. al, 2001). To test my hypothesis, I will be using sympathetic neurons from a domesticated chick embryo, also known as *Gallus gallus.*

This is an important topic to study because mercury ions alter the cell membrane stucture of the developing neuron. They penetrate the cell and bind themselves to newly synthesized tubulin molecules. The mercury ions bind to the binding site reserved for GTP on the beta subunit. Since GTP gives the cell its energy that allows tubulin molecules to attach to one another, mercury ions bound to these sites and prevent tubulin from linking together (Leong et. al, 2001). This leads to the disassembling of tubulin molecules, leaving the neurite stripped of its supporting structure (Leong et. al, 2001). Both the developing neuron and its growth cones collapse. Knowing this can assist in finding the level of toxicity mercury has on developing neurons, and can in turn help in the prevention of diseases and brain dysfunction.

Materials and Methods:

A dissection was performed following the protocol outlined in Morris (2011a). When observing cells the protocol outlined in Morris (2011b) was followed. To stain and observe cells, the protocol as outlined in Morris (2011c) was followed, however mercury was added instead of the L15 buffer for the experimental sample. The control sample remained with just the L15 buffer. This buffer had 15 ng/ml of Nerve Growth Factor 100Umg/ml pen/strep added to it. Before observation the DRG's (dorsal root ganglia) were incubated for 37° C overnight.

For the mercury experiment, a 100nm solution of $HgCl_2$ in Hank's Balanced Salt Solution (HBSS) was made from 10ml of 10mM $HgCl_2$ in 0.05% HCL. For the experimental test it was executed the same way as the control experiment but instead of adding buffer, 1 ml of the 100nm solution of $HgCl_2$ in the HBSS was added to the flow chamber. The solution was left on the flow chamber for ten minutes. The temperature was recorded when each picture was captured and the temperature remained between 31-34°C. When the ten minutes was complete, a second buffer change was done to replace the $HgCl_2$ solution with 1ml of growth medium (done the same way as above). Additionally, in both the experimental and control the pictures were taken at time 0, time 2, time 4, time 6, and time 8. Two trials were done for both the experimental and control, and the pictures/data from the second trial are what was quantified and analyzed.

On the observation day, observations and data were viewed in the ICUC in the Science Center at Wheaton College. The Nikon Eclipse E 200 with phase optics was used. Our images were viewed through a Song DFW-X700 with a 1.0x C-mount. The imaging software that was used was BTW version 6.0b1. The computer that was used was at the "Leo" station, and was a Mac OS X version 10.5.8. Our images were seen through a 40x objective on the phase 2 setting. A picture was taken on the computer (using BTV) at 0, 2, 4, 6, and 8 minutes after exposure to mercury (experimental) or the L15 buffer (control). The slide was heated before taking pictures at each increment using a LASKO ceramic air heater model 754200. The temperature was recorded after each picture was taken and the pictures were saved as "2

min post Hg or 2 min post buffer" etc.

Data was quantified using a program called Image J version 1.40g. Using the protocol packet 11/9/11 67 EMD, the data could be analyzed and quantified. To test growth cone movement, Image J provided a tool that enabled measurements of angle degree. This allowed quantification of how much movement certain growth cones had in buffer and mercury treatments. There were 8 time points in the control that were compared to the corresponding 8 time points in the experimental. This allowed for direct comparison for each time point, one with L15 buffer and one with mercury treatment. When analyzing my data, diverging filopodia that were side by side were observed. Two different sets of filopodia in the experimental and in the control group were observed, so more evidence was able to be obtained. Additionally, the filopodia observed were clearly visible to the human eye, with distinct edges so locating and measuring would be clear. The angle between the two filopida was measured at the point where they are intersected when they are first diverging from the neurite. As each time point progressed, the angle between two filopodia were recorded for two different locations in the control group and were compared to the angle between two filopodia in two different locations of the growth cones in the experimental group at corresponding time points. The angles of the filopodia that are being measured and recorded are labeled with an arrow below in figures 1 and 2. When analyzing, the difference of degrees between the angles at each time point was figured out and the difference was used as an indication for how much the growth cones moved, as seen on Figure's 1 and 2 below. This enabled observations of mercury's effect on the movement of growth cones.

Results:

Below, in figures 1 and 2, are the growth cones used to determine if more movement was evident in the control group versus the experimental group. In figure 3, the average movement of growth cones exposed to mercury and the average movement of growth cones exposed to the L15 buffer were compared. Two diverging filopodia that were side by side in the control and the experimental are labeled by the arrows. The arrows are pointing at the angles in which the degree was measured to track a change in movement of the filopodia in relation to one another. At each time interval, there was significantly more movement in the neurons exposed to the L15 buffer. There was still movement in the growth cones that were exposed to mercury however, not as much as the buffer exposed neurons.

Figure 1

Figure 3 shows a picture of the buffer exposed growth cone (control) after 4 minutes of exposure. The arrows are pointing to the angles that were measured between the two filopodia.

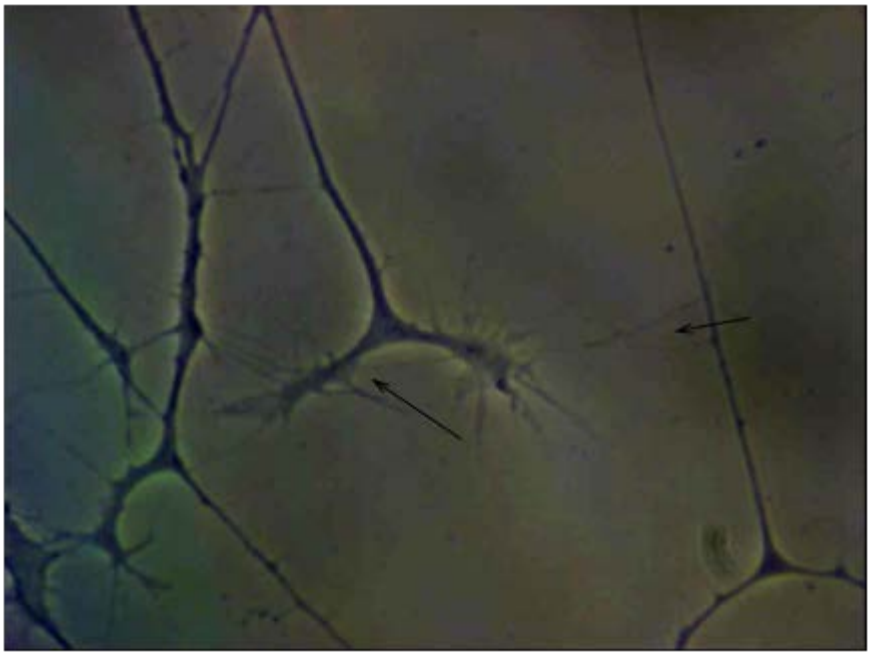

Figure 2

Figure 4 displays the mercury-exposed neuron (experimental) after 4 minutes of exposure. The arrows are pointing to the angles that were measured between the filopodia.

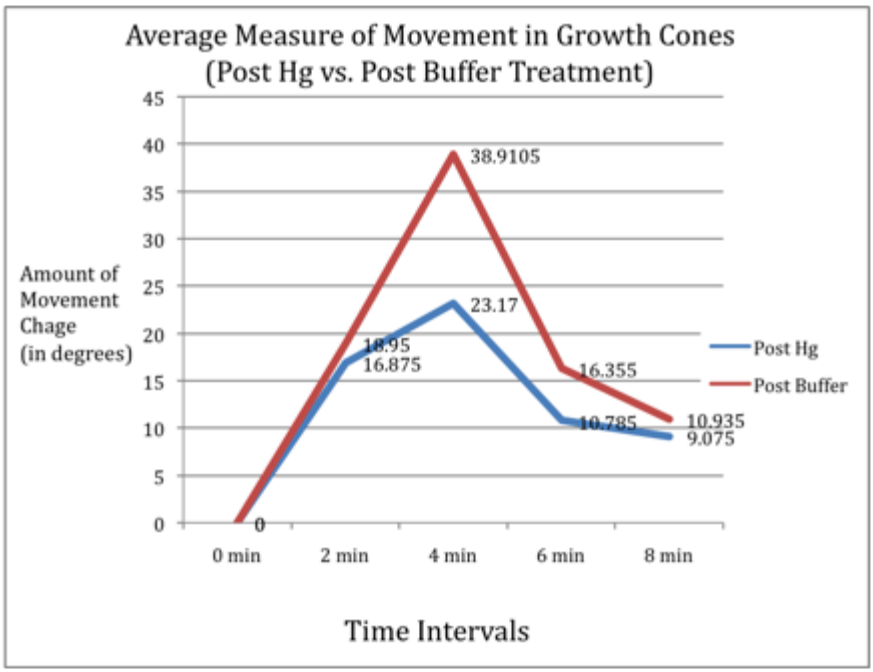

Figure 3

Figure 3 displays the average amount of movement in all growth cones (in degrees). This includes both sets of growth cones examined in the experimental and both sets of growth cones examined in the control. The graph shows significantly more movement in buffer treated growth cones throughout all time intervals. The most prominent evidence of increased movement in post buffer vs. post Hg was shown at approximately 4 minutes.

Discussions and Conclusions:

After experimentation, my results supported my hypothesis. Less growth cone movement took place in neurons exposed to mercury in comparison to neurons exposed to an L15 buffer. The angles between filopodia in the experimental group show overall less movement than those in the control group. This suggests that mercury is toxic to the growth and development of neurons. When the tubulin proteins that link together during normal cell growth to form microtubules, which support the neurite structure, are introduced to a mercury-exposed environment the mercury binds to the newly synthesized molecules and stop the tubulin from linking together (Ngim, 1992). This causes the microtubules to disassemble thus leaving the neurite without key elements of its structural support (Ngim, 1992), and loss of motility of growth cones. This was evident in my experimentation because neurons exposed to mercury displayed less growth cone movement then growth cones exposed to the L15 buffer. The growth cones that were exposed to mercury were affected in a way that their structural support was hindered, thus decreasing in the amount of motility (Ngim, 1992). The L15 buffer did not seem to affect the growth cones as significantly, or at all, because they

showed greater movement. Interestingly, at the four-minute time point, I observed the highest amount of degree change/movement in both the experimental and control groups. This could possibly be because this is the point where the Hg and buffer just start to affect the neuron because at the next time point there is a dramatic decrease in both groups. This would also make sense because until the four-minute time point, the amount of movement is close to the same between the experimental and control groups.

If this experiment had been repeated many times and produced the same results then it would be a good source of evidence that even very low amounts of exposure to mercury can initiate a neurodegenerative process within the brain.

One source of error that arose in my experimentation occurred when the cells were being treated with mercury. When trying to clean up the excess mercury the cover slip moved around a little thus resulting in the inability locate the same cells that were used in the control group. If this experiment were repeated, more data would be gathered, and this would enable me observe more of a trend in the movement of growth cones. Additionally, I would be sure to use the same cells in the control and the experimental so it would be a clearer comparison when questioning impaired movement.

For future experiments I would be interested in testing how mercury exposure affects mitochondria in the neuron and it's ability to form ATP for the cell. This could stem off of my current experiment because energy in the cell is required for movement, therefore if movement is inhibited it must be due to some interference of mitochondria producing ATP. Additionlly, we know that mercury exposure affects microtubules in a negative way, however no evidence of affects of mercury binding to actin have been found. It would be interesting to see if mercury has the same affect on actin that it does on microtubules.

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